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Review article

Detection of oncogenes in chronic pancreatitis

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Background

The pathogenesis of chronic pancreatitis (CP) remains poorly understood. Recently, molecular biology has identified the genetic background for many patients with hereditary CP. In addition, a number of studies have focused on the detection of proto-oncogenes and tumour suppressor gene mutations in the pathogenesis of CP. So far, the use of these mutations (with the exception of mutations causing hereditary CP), as diagnostic and prognostic markers is still controversial.

Discussion

It is well known that the risk of pancreatic cancer in patients with CP, especially the hereditary form, is high. At present, there is insufficient evidence to show a clear relationship between the development of pancreatic cancer and certain mutations. New biotechnological methods, such as DNA array expression analysis, expand our knowledge of the molecular pathogenesis of this disease and may help to develop specific diagnostic, prognostic and therapeutic tools. However, until long-term studies examine the safety and efficacy of certain genetic markers, long-term follow-up of patients with CP who harbour mutations is needed.

Keywords

chronic pancreatitis, oncogenes, mutation, cationic trypsinogen, SPINK-1, CFTR, K-ras, p53, DNA array

Introduction

Chronic pancreatitis (CP) is characterised by progressive inflammatory destruction of the functional parenchyma of the pancreas, resulting in severe exocrine and endocrine insufficiency. Morphologically, prominent features of CP include necrosis, acinar cell destruction and regeneration, severe widespread (intralobular) fibrosis, dysplastic ducts, variable pancreatic duct lesions, foci of proliferating ductal cells, ductular hyperplasia and ductal stones, as well as infiltration of inflammatory cells and alterations of nerves [1, 2]. The incidence of CP is increasing in industrialised countries, and the disease affects 3.5–10 per 100 000 inhabitants in Western countries [3–7], with alcohol abuse being the predominant cause. Although different forms of CP result from different initiating causes, there is evidence to suggest a common pathophysiological pathway in all forms [8–28].

Recently, developments in molecular biology have revealed genetic causes of hereditary CP. Mutations of the cationic trypsinogen gene (PRSS1), as well as of the pancreatic secretory trypsin inhibitor (SPINK-1) gene, seem to predispose people to CP through enhancement of intra-pancreatic trypsin activity and lowering of the threshold for inhibiting trypsin activity [29–45]. In addition, other mutations in several genes have been reported to be implicated in hereditary CP [46–53].

The analysis of gene mutations and gene expression in pancreatic cancer and CP has identified several factors, which are commonly deregulated in both diseases [54–57]. Detection of mutations in the K-ras proto-oncogene and p53 tumour suppressor gene in specimens obtained by needle aspiration, from pure pancreatic juice, duodenal juice, serum or stool, may be clinically useful diagnostic markers for early detection of pancreatic malignancy [58–70]. Although these mutations are considered critical and early events in pancreatic oncogenesis, the role of K-ras and p53 mutations in CP as a precancerous disorder is not completely known. Furthermore, the role of other tumour suppressor genes in the multi-step carcinogenesis remains uncertain.

Epidemiological studies indicate that CP may be associated with pancreatic cancer. Therefore, having a resection procedure before pancreatic cancer develops seems to be more necessary than ever, especially since resection of early pancreatic cancer is the only chance of cure. Thus it is of major importance to identify genetic markers that help to stratify the risk of malignant transformation in CP. Genome-wide gene expression analysis by DNA arrays provides new insights into gene

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function and seems to be another promising diagnostic technique.

Subtypes of chronic pancreatitis

Alcoholic chronic pancreatitis

Increased ethanol consumption is considered to be the most common cause of CP in the Western world. The mortality rate in alcoholic CP approaches 50% within 20–25 years due to malnutrition, severe infections, diabetes, other CP-associated complications, and alcohol- and nicotine-related diseases [6, 7].

Hereditary chronic pancreatitis (HCP)

Hereditary CP, first described by Comfort and Steinberg in 1952, includes diagnostic criteria such as age of onset <20 years and a history of pancreatitis in at least two family members [8, 9]. HCP is characterised by an autosomal dominant model of inheritance, with approximately 80% penetrance and variable expression [10, 11]. Whereas the onset of symptoms is usually in childhood, presentation of the disease may occur from infancy to the fifth or sixth decade of life. Acute attacks range from mild abdominal discomfort to life-threatening episodes, with pancreatic necrosis, splenic vein thrombosis and death [10, 12].

Cystic fibrosis-associated chronic pancreatitis (CFP)

Cystic fibrosis (CF) is the most common autosomal recessive inherited disorder in Caucasians. It presents early in life and is associated with severe chronic progressive pulmonary disease, maldigestion and a shortened life expectancy. Pancreatic duct obstruction induced by a defective chloride channel, as a result of mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, is the cause of pancreatic insufficiency in patients with CF [13].

Tropical pancreatitis

Tropical pancreatitis is reported in tropical belts of the world. It occurs independently of alcohol, nutrition and environment [14, 15]. It is characterised by pancreatic insufficiency, diabetes mellitus and recurrent attacks of pain, commonly with pancreatic calcifications [16, 17]. Children are commonly affected and often die in early adulthood from endocrine and exocrine dysfunction.

Autoimmune chronic pancreatitis

In patients with CP, antibodies against carbonic anhydrase (CA) type I and II have been reported [18, 19]. Carbonic anhydrases are a family of zinc metal enzymes that catalyse the reversible conversion of carbon dioxide to bicarbonate and hydrogen ions. CA II antigens are present in the pancreatic ductal epithelium [20]; therefore, the presence of antibodies against this isoenzyme provides evidence of an immune reaction to a pancreatic target antigen. Moreover, non-specific auto-antibodies, such as antinuclear antibodies and antineutrophil cytoplasmic antibodies, have been found to be present in some patients with CP [19].

Biliary-associated chronic pancreatitis

Biliary lithiasis represents the commonest cause of strictures that involve the main or the secondary pancreatic ducts, leading to stenosis of the papilla of Vater and concomitant progression of CP. However, the role of gallstone disease in the development of CP is still controversial [21, 22]. Nonetheless, recurrent attacks of acute pancreatitis due to gallstones or other causes represent an important and relatively common cause of CP.

Other forms

Less common causes of CP include nutritional factors (hyperlipoproteinaemia), metabolic disturbances (hypercalcaemia), congenital anomalies of the ductal system (pancreas divisum) and acquired pancreatic duct obstruction from strictures secondary to trauma, pseudocysts, mechanical or structural changes of the pancreatic duct sphincter or peri-ampullary tumours, or other rare causes such as choledochal cysts [10, 23–26].

Idiopathic chronic pancreatitis (ICP)

In 15–20% of cases, the cause of CP remains unclear [25, 27].

The mechanisms of tissue destruction and remodelling in CP are difficult to define in the early stages because structural changes are associated with moderate to advanced disease. The diagnostic accuracy of modern imaging techniques – i.e. ERCP, EUS, CT or MRI – has not yet been validated against histopathology as the gold standard [3]. The pancreas is not readily accessible for obtaining tissue specimens, and fine-needle biopsies tend to yield false negative results because the primary lesions of early stage CP are usually focal. Therefore, a clinically

definite diagnosis of CP will be deferred until the disease has reached an advanced stage in which surgical treatment is indicated or in which typical markers of CP, including pancreatic calcifications and/or persistent pancreatic dysfunction, are present. [6]. Despite different causes and forms of CP, the phenotype of chronic pancreatitis is remarkably similar, suggesting a common underlying pathophysiological mechanism.

Molecular genetics in the pathogenesis of hereditary chronic pancreatitis

The theory that inappropriate activation of pancreatic pro-enzymes, especially trypsinogen, within the pancreatic parenchyma leads to auto-digestion and acute attacks of pancreatitis was first reported in 1896 [28]. Under normal conditions, multiple defence mechanisms are known to prevent uncontrolled activation of the digestive enzyme cascade within the pancreas [29, 30]. These include: (a) synthesis of most digestive enzymes as inactive pro-enzymes; (b) compartmentalisation and segregation of pro-enzymes from other subcellular components, within distinct membrane-bound compartments, to prevent their contact with vital cytosolic structures; (c) maintenance of an environmental state of low intracellular calcium; (d) appropriate release of pro-enzymes from pancreatic acinar cells and their activation by intestinal enteropeptidase (this initial activating enzyme hydrolyses trypsinogen to form active trypsin, which subsequently catalyses the conversion of all other pro-enzymes to their active form) and (e) synthesis of pancreatic trypsin inhibitors such as the serine protease inhibitor Kazal type 1 (SPINK1).

SPINK1, also known as pancreatic secretory trypsin inhibitor (PSTI), reversibly inhibits up to 20% of potential available intra-pancreatic trypsin activity by forming a covalent bond between the catalytic serine residue of the enzyme and its own reactive site. The effectiveness of these fail-safe mechanisms is dependent on a balance between the amount of activated trypsinogen and the amount of trypsin that can be inactivated. However, if trypsin activity exceeds the PSTI inhibitory potential, a second line of defence is the ability of trypsin and trypsin-like enzymes such as mesotrypsin to hydrolyse trypsin and other proteases, resulting in a loss of structural integrity and inactivation [30].

The discovery of two heterozygous mutations, R122H and N29I, in the cationic trypsinogen gene in families with HCP constitutes genetic support for the theory that pancreatitis results from inappropriate activation of pancreatic zymogens [28, 31].

Gene mutations in HCP

Cationic trypsinogen gene (PRSS1). Trypsin stabilisation and protection from autolysis appears to play a central role in the pathogenesis of HCP. Mutations in PRSS1 emerge as the cause of most cases of HCP.

In 1996 two researchers, Whitcomb and Le Bodic, independently mapped the HCP gene locus to the long arm of chromosome 7q35 by genome-wide genetic linkage analysis in families with CP [32, 33]. A third researcher, Rowen, had by that time identified the complete DNA sequence of the human beta T-cell receptor in the chromosome domain in which Whitcomb and Le Bodic located the HCP gene [34], leading to the discovery of eight other pancreatic trypsinogen genes. This discovery enabled Whitcomb to design specific primers for amplifying the cationic and anionic trypsinogen genes and to identify two HCP-associated mutations in PRSS1 [31]. One mutation was found in exon 3, where a codon CGC was changed into CAC, resulting in replacement of the amino acid arginine (R) by the amino acid histidine (H) at position 122 in the gene product, thus eliminating the initial site for trypsin hydrolysis. Gorry *et al.* identified the second and less frequent disease-specific mutation in PRSS1 resulting in a codon AAC changing to ATC in exon 2 of the gene, which substitutes the hydrophobic amino acid isoleucine (I) at position 29 for the more polar asparagine (N) [35]. These mutations are now named R122H and N29I [36].

Other mutations in PRSS1 have also been reported to be involved in the pathogenesis of CP. In a study of 44 children with CP, a C to T transition in exon 2, leading to replacement of alanine by valine at codon 16 (A16V), was detected in four unrelated patients [37]. Three of these patients had no family history of CP, although the mutation was inherited in all cases from one parent. However, only one of seven first-degree relatives with the A16V mutation was affected, indicating a low penetrance of this mutation in the pathogenesis of HCP, in contrast to the above-mentioned R122H and N29I mutations.

SPINK-1 gene. Mutations in the SPINK-1 gene result in an impaired function of PSTI, leading to diminished inhibition of trypsin in pancreatic acinar cells. Pancreatitis may therefore be the result of an imbalance of proteases and their inhibitors within the pancreatic parenchyma.

Witt and co-workers found an A to G transition resulting in substitution of asparagine by serine at codon 34 in exon 3 (N34S) in the gene product PSTI, among 18 out of 97 children (19%) with ICP [38], a percentage that was significantly higher than in a control group. Six patients (6%) were homozygous for this mutation, but no phenotypic differences between heterozygous and homozygous N34S patients were observed. The high frequency of N34S in CP has been confirmed by others [39–42].

The second and also less frequent mutation was replacement of proline (P) by serine (S) at position 55 of PSTI. Chen *et al.* [43] detected P55S in 1 of 44 patients with CP and in 2 of 200 control subjects. By contrast, Witt did not find P55S in any of the 96 patients with CP, but found it in 1 out of 52 control individuals. Therefore, mutation in P55S should be classified more as a polymorphism than a disease-causing mutation [44]. However, the frequency of mutations in the SPINK-1 gene in the general population does not exceed 1%, or 1:40 000, for N34S homozygotes [45]. Although the percentage is significantly high in ICP patients, it is still unclear why the majority of N34S carriers do not develop CP.

CFTR gene. Mutations in the CFTR gene cause an impaired regulation of the cyclic adenosine monophosphate (AMP)-regulated chloride channels, leading to impairment of various organs, including the lung, pancreas and vas deferens. Especially, two mutations of the CFTR gene, R117H and the 5T allele, are associated with an enhanced relative risk for CP [46–48].

In a group of 27 patients with ICP, Cohn and coworkers [46] found an 11 times higher than expected rate in the frequency of one CFTR mutation and an 80 times higher than expected rate in the frequency of two CFTR mutations. Sharer and colleagues [47] studied 134 consecutive patients with CP and also found a 2.5 times higher frequency of one CFTR mutation than the expected rate in the general population. The last observation has been confirmed by others [49]. Almost 800 CFTR mutations have been reported so far, yet their relative frequencies in CF do not necessarily match those of CP [13].

Other mutations. Multiple mutations (an A16V mutation of the PRSS1 plus the CFTR-R117H mutation) have been reported in a family with episodes of acute and chronic pancreatitis linked to polygenic pancreatitis [31]. Moreover, other mutations in well characterised genes have been reported to be implicated in CP. Familiar hyperparathyroidism with hypercalcaemia and mutations in lipoprotein lipase gene and/or apolipoprotein C-II, for example, have been shown to be associated with CP [50–53].

Oncogenes and tumour suppressor genes in chronic pancreatitis

Mutations involve genes, called proto-oncogenes, such as ras genes (N-ras, H-ras and K-ras) and tumour suppressor genes (p53, p16, Smad4) that are engaged in the control of cell growth, differentiation and death. When growth factors bind to tyrosine kinase receptors, ras proto-oncogenes become activated by an exchange of the associated guanine nucleotide GDP by GTP, which acts as a molecule in the intracellular signal transduction process, initiating downstream cascades. In case of K-ras mutations, for example, substitution of glycine by any other amino acid residue (with the exception of proline) results in a reduced rate of guanosine triphosphate (GTP) hydrolysis and in resistance to the inhibitory effects of GTPase-activating proteins (GAPs). The mutant K-ras proteins are therefore locked into an active GTP-bound state and transmit a constitutive growth signal to the nucleus [54–57].

On the other hand, tumour suppressor genes such as p-53 encode a protein that suppresses cell division and induces apoptosis under special circumstances. When a tumour suppressor gene is lost or damaged, the control it exerts over cell growth and replication is lost, thereby triggering cancer. Apart from being involved in the process of pancreatic carcinogenesis, these genetic alterations are generally believed to contribute to the pathogenesis of CP as well.

Detection of oncogenes in tumour suppressor genes in chronic pancreatitis

Fine-needle aspiration. Fine-needle aspiration (FNA), guided by endoscopic ultrasonography (EUS), ultrason-

ography (US) or computed tomography (CT), is used for the diagnosis and staging of pancreatic cancer. US or CT may not be able to differentiate between focal CP and cancer, leading to lower specificity rates, whereas EUS-FNA has been reported to be the superior technique, as it employs a needle track with a shorter length, uses small diameters of needles and has easier access to small intra-pancreatic masses [58, 59]. However, histopathological diagnosis is often insufficient due to the small amount of aspirated specimen. Combinations of pathological diagnoses and analysis for mutant K-ras genes are used to improve the accuracy of diagnosis [60].

Pancreatic duct brushings. Tight pancreatic ductal strictures and obstructed ducts are difficult or impossible to brush. However, Sawada *et al.* [61] succeeded in brushing 100% of pancreatic strictures using a thinner brush. In the case of ductal obstruction, a biopsy specimen can be obtained by using highly flexible forceps, a technique with which Kubota *et al.* [62] achieved a sensitivity of 71% without complications. The sensitivity of pancreatic brush cytology can be coupled with salvage cytology (i.e. concentration of the collected material), as has been shown in some studies [63].

Pancreatic-duodenal juice. The duodenal juice contains cell components originating from the normal pancreatic duct, from any hyperplastic or malignant lesion of the pancreatic duct, and from other regions such as the biliary and gastrointestinal tracts. Collection of duodenal juice during a secretin test is an easier technique than endoscopic collection of pure pancreatic juice, possibly because of a disruption of the ductal epithelium with hyperplastic change that leads to a higher positive rate for K-ras mutations in patients with CP [64]. Thus, the positive rate for K-ras mutations is much lower in the duodenal juice than in the pure pancreatic juice.

Peripheral blood. A potential non-invasive source of DNA in pancreatic cancer patients is the serum. The higher specificity of K-ras mutation detection in the serum as compared with tissue or pancreatic juice, where DNA concentration is higher, leads to the use of genetic analysis of serum DNA in conjunction with CA 19-9 as an additional diagnostic tool [65].

Stool. Testing for K-ras status in the stool is a non-invasive method with no risk of complications and is thought to serve as a candidate test for screening persons at increased risk for pancreatic tumours, such as elderly persons, those with predisposing hereditary disorders (e.g. BRAC2 mutation carriers [66]) and patients with CP [67]. However, the diagnostic value of K-ras genotyping in stool has been compared to that of tissue samples and the serum tumour markers CA 19-9 and CEA in pancreatic diseases, while K-ras genotyping has been found to lack the specificity to discriminate malignant pancreatic disease from chronic inflammation [68–70].

Molecular markers for differentiating chronic pancreatitis from pancreatic cancer

K-ras gene

K-ras has been found to be mutated in the vast majority of pancreatic adenocarcinomas. Abnormal activation of K-ras, which is located on chromosome 12p13, is due to permanently activated proteins that result from point mutations in codons 12, 13 or 61. The incidence of mutation at codon 12 is definitely higher when compared with mutations at codon 13 or 61 in pancreatic adenocarcinoma [54–57]. Although point mutations at codon 12 of the K-ras oncogene have been found in 75–100% of pancreatic cancer tissues, K-ras mutations are present in pancreatic carcinoma in situ as well, which makes early tumour detection possible [57, 71–74]. Previous studies reported K-ras mutations also in hyperplastic mucous cells of the pancreas with chronic inflammation [70, 75]. This information has been recognised as a new and promising approach to the diagnosis of PC and its relationship with pancreatic adenocarcinoma.

The incidence of K-ras mutations in duct lesions in CP is uncertain, ranging between 0 and 60% [76–81] due to differences in sampling, DNA extraction or polymerase chain reaction (PCR) methods. However, in two recent studies no single K-ras mutation in pancreatic tissue specimens from patients with CP was reported [60, 82]. Although K-ras mutations have been identified in pure pancreatic juice collected endoscopically from patients with pancreatic cancer, such mutations were not identified in patients with CP in these studies, probably because of the small number of patients examined [83–85]. In another study [64], mutations of the K-ras codon 12 were found in the duodenal juice in 63% of patients with pancreatic cancer but only in 1 patient with CP out of 41 patients with benign pancreatic disorders.

Additionally, K-ras mutations have also been detected in juice samples from patients with CP in the absence of pancreatic carcinoma [86]. Interestingly, during follow-up of 12 patients with CP and ras mutation, van Laethem [87] found two cancers at 18 and 24 months. By contrast, a Japanese study [88] detected no cancers in 20 patients with CP over a mean follow-up period of 78 months.

p53 gene

The most common and widely distributed tumour suppressor gene, p53, located on chromosome 17p13, encodes a 53-kDa nuclear phosphoprotein (p53 protein) and plays a central role in genetic stability and cell survival. Mutations of the p53 gene have been found in 40–76% of pancreatic cancers [72, 89–92], with the majority of them located in exons 5–8. Due to an increased half-life and accumulation of the p53 protein, caused by p53 mutation, p53 protein concentrations in serum may be an additional tumour marker in pancreatic cancer patients [93]. However, the correlation of p53 over-expression with survival in pancreatic cancer is controversial [94–96].

By contrast, the frequency of p53 mutations in patients with CP is markedly less, varying from 0 to 10% depending on various biological samples. Gansauge and co-workers [97] reported alterations in the p53 gene in tissue specimens in 8 of 80 patients with CP who underwent resection, whereas another study reported a zero incidence [98]. Löhr *et al.* [81] evaluated the presence of p53 in pancreatic juice samples of 66 CP patients without evidence of pancreatic carcinoma and found that 7.5% had p53 mutations, a finding that has been confirmed by others [88, 99]. There has been no evidence of increased p53 expression indicating p53 mutations in serum and pancreatic brush cytology in CP patients [93, 100]. Although the number of patients with CP included in these studies is small, it is obvious that identification of a p53 mutation seems to be more specific for the diagnosis of pancreatic cancer than K-ras mutations.

p16 gene (p16INK4a)

The p16INK4a tumour suppressor gene, located on chromosome 9p21, encodes a cyclin-dependent kinase inhibitor, preferentially binding to CDK4 and CDK6, preventing the coupling of those kinases with D-type cyclins and thus the activating phosphorylation of Rb. Therefore, the functional impairment of p16INK4a is suggested to lead to uncontrolled cell cycle progression and neoplastic transformation [101, 102].

Loss of p16 expression in 87% of cases was consistent with the high frequency of p16 mutation in pancreatic cancer [103–106]. Loss of expression of p16 was also found in 60 of 126 microdissected intraductal lesions, and three times more often in atypical lesions than in non-atypical lesions, suggesting that it occurs more frequently in higher grade duct lesions [107, 108]. Expression of the p16 gene product was investigated in paraffin-embedded tissue using a monoclonal antibody against p16 protein. All six cases of normal pancreas and all but 1 of 20 cases of CP expressed p16 protein, whereas 37.5% (3 of 8) of cystadenomas and 41.9% (26 of 62) of PCs lost p16 expression [109]. Nonetheless, the incidence of p16 mutations in pure pancreatic fluids obtained from patients with CP varies greatly (0–50%) [99, 106].

Smad4 gene

The Smad4 gene (DPC4) is a tumour suppressor gene located on chromosome 18q21. It belongs to the Smad family of proteins, which plays a critical role in signal transduction through the TGF-β superfamily of cell surface receptors. Under-expression of the TGF-β signalling pathway, which exhibits growth, may be a positive prognostic factor in pancreatic adenocarcinoma [110], as bi-allelic inactivation of the Smad4 gene is reported in approximately 50% of cases [111]. In addition, inactivation of DPC4 occurs more frequently in tumour-derived cell lines than in primary pancreatic adenocarcinomas [112].

However, inactivation of the Smad4 gene in pancreatic juice samples of 20 patients with CP was given at a high frequency of 58%, which is surprisingly higher than the 36% found in patients with pancreatic cancer [99]. The prognostic relevance of p16 and Smad4 as genetic markers remains poorly defined due to a lack of reports and small series of cases. More thorough analysis is needed to confirm their role in early detection of carcinogenesis in CP.

DNA array technology

DNA array expression analysis is a powerful new technology that allows the simultaneous analysis of the

expression of multiple genes. Friess and co-workers [113] were the first to investigate differential gene expression using DNA array in CP. Eleven signature genes were identified for the pancreas which are not present in human colon, liver, prostate, lung or lymphatic tissue. This analysis of 5600 human genes also revealed increased expression of 157 genes in CP compared with the normal pancreas; 152 of those genes were simultaneously increased in PC and only 5 genes were uniquely increased in CP. Additionally, 34 genes decreased their expression in CP compared with the normal pancreas and PC. However, these correlations may simply reflect a pathologic pancreas rather than linked pathologic pathways, as CP-like changes are also present in almost all PC samples [113]. Nevertheless, these results provide new insights into the pathological changes of CP by identifying alterations in gene expression patterns that might serve as targets for new diagnostic tools and disease-specific therapy.

Chronic pancreatitis as a risk factor for pancreatic cancer

Chronic pancreatitis, especially the hereditary form, has been proposed as an independent risk factor for the development of pancreatic cancer, on the basis of large epidemiological studies [114, 115]. In a multi-centre study of 2015 patients with CP, Lowenfels *et al.* [116] identified 56 cancers during a mean (±SD) follow-up of 7.4 ± 6.2 years. In a cohort study, Malka and colleagues [117] followed up 373 patients with proven CP over a median of 9.2 years and also reported a significantly increased overall risk for pancreatic cancer (standardised incidence ratio 26.7).

The risk of developing pancreatic cancer in conjunction with CP is believed to be related to the age of the patient and the duration of CP. In a study of 1552 patients with CP, the relative risk of the development of pancreatic cancer was more than three times greater for patients over the age of 60 years compared with younger patients [116]. The estimated cumulative risk in patients with HCP was found to be 40% for patients aged 40–70 years, while it was negligible below the age of 40 years [115]. However, it is unclear whether that high risk is caused by the prolonged duration of CP, as in the hereditary form, or whether there are age-specific differences in the response of the pancreas to CP.

In tropical CP, the risk of pancreatic cancer is much higher, affecting 8.3% of patients [118], with a roughly 100-fold increased incidence compared with the general population [119]. Similar rates were observed for patients suffering from CF-related CP [120, 121].

Smoking has been shown to compound the risk of pancreatic cancer in CP. In a multivariate analysis of 497 patients with HP, Lowenfels *et al.* [122] found that smoking independently doubled the risk of pancreatic cancer, which even developed 20 years earlier in smokers compared with non-smokers and accounted for approximately 25–30% of all pancreatic tumours.

Genetic studies of oncogene mutations are useful in expanding our knowledge on the relationship between CP and pancreatic adenocarcinoma. In a prospective study of 76 patients with CP (59 alcoholic, 15 idiopathic and 2 hereditary), van Laethem *et al.* [73] examined K-ras mutations in collected ductal brushings during therapeutic ERCP and after long-term follow-up, every 6–12 months. K-ras (codon 12) mutations – GAT and GTT in the majority of the cases – were found in 25% of the patients, quite similar to those found in adenocarcinoma. There was no relationship between the presence of mutant K-ras and the aetiology of CP (alcoholic: 20% of mutant K-ras; idiopathic: 30%; hereditary: 50%), the duration of the disease before the analysis, the presence of acute attacks, the existence of pancreas divisum or the presence of a pancreatic stent.

Ductal mucinous cell-epithelial hyperplasia, metaplasia and dysplasia, and K-ras mutations have been described in pancreatic cancer [70], in the pancreas with CP [70, 75], and in the pancreas of a patient with a family history of pancreatic carcinoma [123], leading to the hypothesis that K-ras mutations and pancreatic ductal cell hyperplasia may also influence the magnitude of the increase in risk of pancreatic cancer. Although hyperplasia is also frequently observed in the normal pancreas, carcinogenesis is thought to be a multi-step process which is characterised by accumulation of genetic alterations.

Conclusion

In conclusion, the significance of K-ras or p53 mutations in CP has not yet been clearly defined. However, the finding of these mutations in different samples of patients with CP might indicate the necessity for a resection

procedure before the development of pancreatic cancer. Thereby, point mutations in the K-ras gene or p53 might be used in screening protocols for high-risk cases of CP. New technologies, such as DNA arrays, provide a systematic way to survey concomitant RNA expression of a large number of genes and could help in understanding and evaluating the pathology of CP. In the future, specific alterations identified by DNA array analysis will serve as targets for new diagnostic strategies in CP. Certain alterations will also enable the clinician to predict the course of the disease at an early stage. Nevertheless, the mechanisms underlying the risk of pancreatic cancer in CP patients are still unclear. Further cohort studies must be performed, including long-term follow-up of patients with CP-harbouring mutations, to determine the incidence of pancreatic cancer in these patients.

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CASE REPORTS

Like many journals, *HPB* now receives more Case Reports than it can accept for publication. The Editorial Board of the Journal discussed this matter in detail at a recent meeting held during the European Congress of the IHPBA in Istanbul, Turkey. It was generally agreed that it would still be appropriate to include in each issue a few succinct descriptions of clinical cases that are of particular interest, especially if they are well illustrated. However, an issue composed largely of such material would not be of interest to the majority of readers, nor would it assist in our objective of having the Journal fully indexed. A particularly unattractive format is the case report with a review of the literature, in which a single case is used as the justification for an exhaustive overview of the literature containing upwards of 30 references. The editorial policy for *HPB* will therefore be to increase the number of original and review articles at the expense of Case Reports, which will become restricted to 25 or cent of less of the pages allocated for each future issue.

Contributors to *HPB* must expect an increasingly high rejection rate for Case Reports over the next year or two. To ensure that there is still an opportunity to publish the best of these articles, the Editorial Board has decided to introduce rather more stringent criteria for acceptance. From now on Case Reports submitted to the Journal must conform to the following guidelines:

A maximum of 500 words of text to include the Abstract
A short structured Abstract is required: Background, Case outline, Discussion.
No more than two figures.
No more than ten references.

Please note that as for all material submitted to the Journal, I need to receive three paper copies of the manuscripts and figures plus one copy of the article on diskette. In addition, please ensure that the covering letter includes all the authors' signatures to demonstrate that the decision to submit the article for publication in *HPB* is unanimous. Regrettably, any article that does not conform to these guidelines will not be considered for publication.

Robin Williamson
Editor